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New species of *Lemophagus* Townes, 1965 (Hymenoptera, Ichneumonidae, Campopleginae) from India reared as a larval parasitoid of *Crioceris nigroornata* Clarke, 1866 (Coleoptera, Chrysomelidae)

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Abstract

The campoplegine genus *Lemophagus* is reported for the first time from India, along with the description of a new species, *L. larvivorus* **sp. nov.** The specimens were reared as larval parasitoids of the chrysomelid beetle *Crioceris nigroornata*. The new species is compared with all extant species, and its validity is further supported by molecular data. A concatenated maximum likelihood tree of COI+28S gene fragments is presented including species with available molecular data.

Keywords

Beetle parasitoid, molecular phylogeny, new distribution record, Oriental region

Introduction

Campopleginae is one of the largest subfamilies of Ichneumonidae, comprising more than 2,100 species in 66 genera (Broad et al. 2018). This subfamily is considered one of the two major radiations of parasitoids which use Lepidoptera as hosts (Yu et al.

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2016; Broad et al. 2018). Campopleginae is consistently recovered as the sister group to Cremastinae (Quicke et al. 2009; Bennett et al. 2019). Most species of Campopleginae develop as koinobiont endoparasitoids of Lepidoptera (Broad et al. 2018). Several small genera are associated with exposed, subterranean, and wood-boring Coleoptera such as *Bathyplectes* Förster (Curculionidae), *Lemophagus* Townes and *Nepiesta* Förster (Chrysomelidae), *Lathroplex* Förster (Dermestidae), *Pyracmon* Holmgren (Elateridae and Artematopodidae), and *Rhimphoctona* Förster (Cerambycidae and Curculionidae) (Jolivet 1950; Jolivet and Théodoridès 1952; Horstmann 1974, 1978, 1980; Barron and Walley 1983; Sanborne 1986; Haye and Kenis 2004).

The campoplegine fauna of India is heavily understudied, with only 192 species reported from 21 genera (Yu et al. 2016; Vas 2020). This assemblage is largely dominated by genera such as *Dusona* Cameron, *Casinaria* Holmgren, *Campoplex* Gravenhorst, *Eriborus* Förster, and *Venturia* Schrottky (Gupta and Gupta 1977; Gupta and Maheswary 1977). Studies on the biological associations of Indian Campopleginae are scarce. The genus *Lemophagus* is considered a specialist parasitoid of chrysomelid beetles and includes ten species distributed across the Nearctic, Oriental, and Palaearctic regions (Horstmann 2004; Yu et al. 2016; Vas et al. 2022; Han et al. 2024). Four species, *L. curtus* Townes, *L. japonicas* Sonan, *L. nanus* Han et al. and *L. pulcher* (Szépligeti), are known from the Oriental region (Sonan 1930; Yu et al. 2016; Han et al. 2024).

The present study reports the first distribution record of the genus *Lemophagus* from India, reared from *Crioceris nigroornata* (Chrysomelidae), along with the description of a new species, *L. larvivorus* sp. nov. The new species is illustrated and compared with all extant species. Additionally, its validity has been confirmed through molecular analysis of cytochrome c oxidase I (COI) + 28S rRNA D2-D3 expansion region (28S) sequences. A maximum likelihood tree is reconstructed using available sequences of *Lemophagus* species. The biological associations of *Lemophagus* are briefly discussed.

Materials and methods

Specimens were reared from the larval stages of *C. nigroornata* feeding on *Asparagus racemosus* from Lucknow, Uttar Pradesh, India (26°53'38"N, 80°59'2"E, 120 m.a.s.l.). Images were taken with Keyence VHX-6000 digital microscope. Holotype is deposited in National Zoological Collections of the Zoological Survey of India, Western Ghats Regional Centre, Kozhikode, Kerala, India (**ZSIK**) and paratypes are deposited in Department of Zoology, University of Calicut, Malappuram, Kerala, India (DZUC). Morphological terminology follows Broad et al. (2018) and cuticular sculpture follows Harris (1979).

Abbreviations used

OOL ocular-ocellar line;
POL postocellar line.

Sequencing and phylogenetic analysis

Sequences of two molecular markers (cytochrome oxidase I and 28S rRNA) were obtained based on standard protocols as described in Hebert et al. (2003), Park et al. (2010), Magnacca and Brown (2010), Belshaw and Quicke (1997) and Quicke et al. (2021). Polymerase chain reaction, amplification and DNA sequencing were performed following standard protocols for DNA extraction, PCR and sequencing (Hajibabaei et al. 2005; Ivanova et al. 2006; de Waard et al. 2008).

Alignment of COI gene (657 bp) was trivial as there were no indels and 28S gene (660 bp) was aligned using the EINS-i strategy on the MAFFT web server (Katoh et al. 2019). A single female of the new species was barcoded and representative sequences of available *Lemophagus* species were added to the dataset. Single sequences of *L. crioceritor* Aubert, *L. curtus* Townes, and *L. pulcher* (Szépligeti) were obtained from BOLD and sequence of *L. errabundus* (Gravenhorst) was obtained from NCBI respectively. A sequence of *Olesicampe* sp. was obtained from BOLD to serve as an outgroup for rooting purposes. We included COI sequences for all four species (*L. crioceritor* Aubert, *L. curtus* Townes, *L. errabundus* (Gravenhorst), *L. pulcher* (Szépligeti) and *L. larvivorus* sp. nov.) whereas 28S sequences were not found for two species, *L. crioceritor* and *L. curtus* (Table 1).

The best-fit nucleotide substitution model for maximum likelihood was selected for both genes based on the corrected Akaike Information Criterion (AICc) in PartitionFinder 2.0 (Lanfear et al. 2017). The GTR+G substitution model was assigned to the first codon position, and the HKY+G model was assigned to the second and third codon positions for COI. The GTR substitution model was assigned for 28S. Phylogenetic analysis was conducted using Maximum Likelihood (ML) for the concatenated dataset of COI and 28S gene fragments (1317 bp) (Suppl. material 1), implemented with IQTREE v2.1.3 (Minh et al. 2020). Branch support was evaluated with 1,000 ultrafast bootstraps (Hoang et al. 2018). The resulting phylogenetic tree was visualized using FigTree v. 1.4.4 (Rambaut 2018).

The incidence of *Crioceris nigroornata* on *Asparagus racemosus* Willd. was observed during October 2020. A total of 80 larvae (20 larvae per week for four consecutive weeks) were collected to study the associated parasitoids while surveying insect pests of

Table 1. Specimens used for molecular analysis with their provenances, GenBank accession numbers and BOLD information for sequence analysed.

Species	Provenance	BOLD sample ID	BOLD process id	GenBank accession no.	
				COI	28S
Lemophagus larvivorus sp. nov.	India	CCDB-44298-H09	BBTH5030-22	PV263161	PV264849
Lemophagus crioceritor	Canada	08TTML-0353	TTMHY353-08	HM417120	–
Lemophagus curtus	Germany	CollHH3450	DTIII3258-22	–	–
Lemophagus errabundus	Germany	–	–	EU378411	EU378411
Lemophagus pulcher	Germany	–	–	MN729650	EU378412
Oleisecampe sp.	USA	CNC 422491	HYCNL020-19	MK959452	MK851129

A. racemosus. The collected larvae along with parts of the host plant were kept individually in insect rearing cages under laboratory conditions (25 ± 5 °C and 65 ± 5 % RH 12L: 12 D photoperiod). Fresh shoots of *A. racemosus* were provided regularly to the larvae until emergence of either parasitoids or adult beetles.

Results

Taxonomy

***Lemophagus larvivorus* sp. nov.**
https://zoobank.org/314B4531-4500-4991-AA8C-CEC82CBED862
Figs 1, 2

Material examined. ***Holotype***, female, India • Uttar Pradesh, Lucknow, CIMAP, 25.x.2020, reared from *Crioceris nigroornata*, coll. Santosh C. Kedar (ZSIK: ZSI/WGRC/I.R.-INV.28945), ***Paratypes*** • 5 females 2 males same data as holotype, one paratype female with the following data, Voucher code: CCDB-44298-H09 Process id: BBTH5030-22 BOLD BIN number: BOLD:AAM7398 Genbank accession number, COI: PV263161, 28S: PV264849 (DZUC).

Description. Holotype, female. Body length 4.4 mm, fore wing 2.9 mm.

Head. Head 1.1×, 2.2× as wide as long in anterior and dorsal view, respectively. Face rugose-punctate with longitudinal striae, setose, 2.0× as wide as long (Fig. 1B). Clypeal sulcus absent (Fig. 1B). Clypeus slightly convex in lateral view, relatively wide in frontal view, punctate, setose (Fig. 1A, B, E). Malar space rugose, setose, 0.8× as long as basal width of mandible (Fig. 1D, E). Mandible broad, upper tooth slightly longer than lower tooth (Fig. 1D). Gena irregularly punctate, setose (Fig. 1A, E). Eye glabrous, 1.3× as long as temple dorsally. Frons slightly depressed behind antennal sockets, transversely striate-rugose with complete median longitudinal carina, setose (Fig. 1B). OOL: diameter of posterior ocellus: POL = 1.7: 1.0: 2.0. Vertex granulate (Fig. 1C). Occipital carina complete, joining with hypostomal carina at base of mandible. Antenna with 26 segments. Scape cylindrical. First flagellomere 1.3× as long as second, first and second flagellomere 3.8, 2.3× as long as wide respectively.

Mesosoma. Mesosoma 1.3× as long as high (Fig. 1A, E). Pronotum with transverse groove dorsally, laterally with indistinctly crenulate groove (Fig. 1C). Mesoscutum rugose-punctate, transversely striate medio-posteriorly, setose (Fig. 1F). Notauli absent, notaular area slightly indented by elongated rugose sculpture (Fig. 1F). Scuto-scutellar groove with indistinct striae (Fig. 1F). Scutellum rugose, setose with lateral carina on anterior 1/2 (Fig. 1F). Epicnemial carina not reaching anterior edge of mesopleuron (Fig. 1E). Mesopleuron punctate, obliquely striate medially, speculum coriaceous, episternal scrobe impressed, mesopleural furrow crenulated (Fig. 1E). Posterior transverse carina of the mesosternum complete (Fig. 1D). Metapleuron faintly

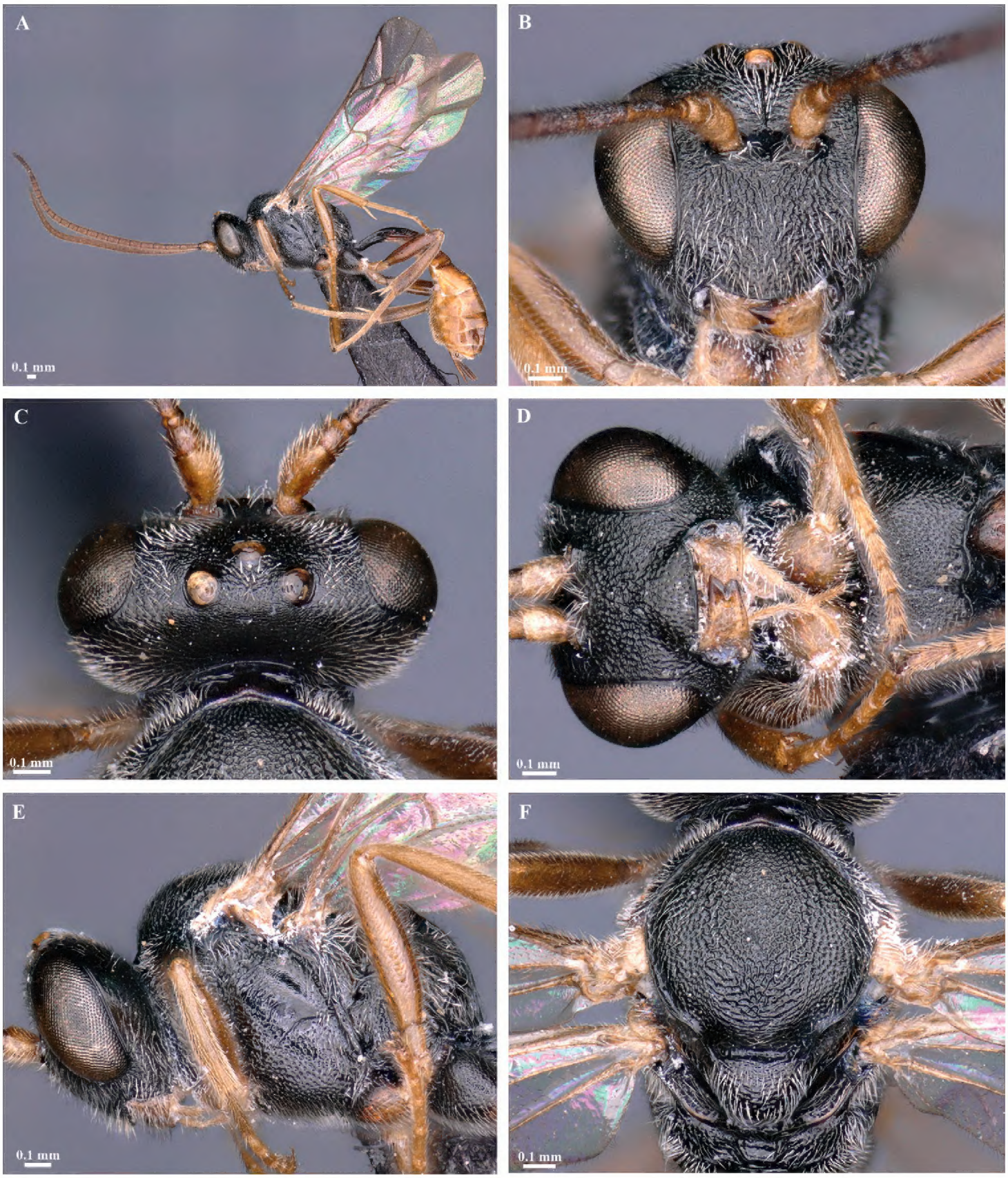

Figure 1. *Lemophagus larvivorus* sp. nov., holotype, female. **A** Habitus, lateral view **B** head, anterior view **C** head, dorsal view **D** head, ventral view **E** head and mesosoma, lateral view **F** mesosoma, dorsal view.

rugose (Fig. 1E). Pleural carina complete (Fig. 1E). Metanotum medially with longitudinal striae (Fig. 2A). Propodeum rugose, setose, area basalis trapezoid, area superomedia and area petiolaris confluent, area superomedia pentagonal, 1.4× as long as wide, narrowing posteriorly, costula indistinct, area petiolaris strongly widening posteriorly, lateral section of posterior transverse carina short, propodeal spiracle slightly oval connected with pleural carina by a carina (Fig. 2A).

Wings. Hyaline. Fore wing (Figs 1A, 2F): Pterostigma 4.0× as long as wide. Vein 1cu-a interstitial (Fig. 2F). Areolet closed, short-stalked (Fig. 2F). Hind wing: Nervellus not intercepted (Fig. 2F). Vein RA with five hamuli (Fig. 2F).

Legs. Hind coxa punctate (Figs 1A, 2B). Hind femur 4.4× as long as wide. Hind tibia 5.4× as long as wide. Hind basitarsus 5.2× as long as wide. Longest hind tibial spur 0.6× as long as hind basitarsus.

Metasoma. Metasoma 1.4× as long as head and mesosoma combined, laterally compressed (Figs 1A, 2B). First metasomal tergite 2.5× as long as wide, transverse in cross section, depressed medio-basally, latero-median carina present extend till spiracle, glymma small, dorso- and ventro-lateral carina present, incomplete, faintly rugose subbasally, granulate subapically and postero-laterally, smooth postero-medially, setose postero-laterally, glabrous postero-medially (Fig. 2B, C). Second metasomal tergite 0.9× as long as wide, granulate, setose, thyridium oval (Fig. 2D). Third metasomal tergite as long as wide, faintly granulate, setose (Fig. 2D). Metasomal tergites 4–7 finely sculptured, setose (Fig. 2D). Ovipositor sheath setose, 0.4× as long as hind tibia (Fig. 2E). Ovipositor with dorsal notch quite far situated from apex as characteristic for *Lemophagus* species (Fig. 2E).

Colour. Head black except mandible yellow, apex of mandible brown, scape and pedicel yellowish brown, flagellomeres brown, maxillary and labial palps yellowish brown. Mesosoma black except tegula yellow. Fore leg yellowish brown, mid leg yellowish brown except mid coxa brown basally, hind coxa black yellowish brown apically, hind trochanter yellowish brown, hind femur and tibia reddish brown, hind tarsus yellowish brown. Wing venation and pterostigma yellowish brown. Metasoma yellowish brown except first metasomal tergite black, second metasomal tergite black except posterior margin reddish brown, third metasomal tergite black anteriorly, rest of the tergite reddish brown. Ovipositor sheath brown.

Male. Same as female.

Biology. Larval parasitoid of *Crioceris nigroornata* Clark, 1866 (Coleoptera: Chrysomelidae) (Fig. 4).

Etymology. The species epithet *larvivorus* is derived from the Latin words *larva* (meaning "larva") and *vorus* (meaning "devourer" or "eater"). It refers to the specialization in attacking and developing within the larval stage of its host. Gender is masculine form of an adjective.

Distribution. Oriental (India).

Diagnosis. The new species can be reliably identified by the following character states in combination: face rugose-punctate with longitudinal striae; clypeus punctate; malar space rugose; occipital carina joining with hypostomal carina at base of mandible; mid-longitudinal carina of frons associated with transverse striations; mesoscutum punctate laterally, rugose on notaular area, rugulose medio-posteriorly, transversely striate posteriorly; speculum coriaceous; first metasomal tergite with a distinct small glymma.

Identification. The new species can be distinguished from the nearest species by the following combination of characters; first metasomal tergite with distinct small glymma, speculum coriaceous, mesoscutum with transverse striae posteriorly. Out of

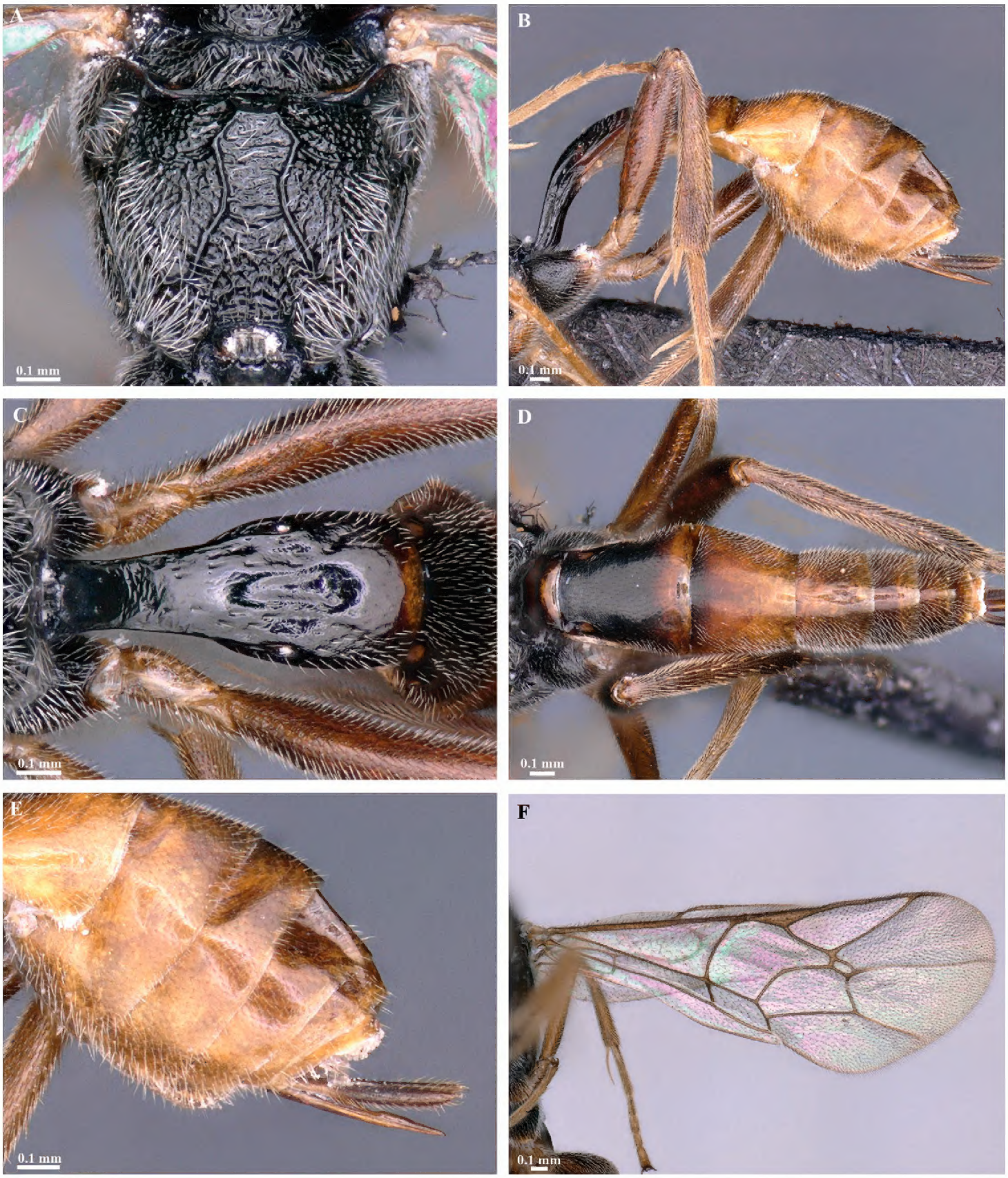

Figure 2. *Lemophagus larvivorus* sp. nov., holotype, female. **A** Propodeum, dorsal view **B** metasoma, lateral view **C** first metasomal tergite, dorsal view **D** metasomal tergites 2–7, dorsal view **E** apex of metasoma, lateral view **F** fore wing.

the 10 extant species only four species were reported from the Oriental region. The new species can be separated from all Oriental species by the presence of short glymma on first metasomal tergite. Based on the molecular and morphological data, the new species comes close to *L. curtus*. However, the new species can be distinguished from *L. curtus* by the following differences; first metasomal tergite with distinct, small glymma (without glymma in *L. curtus*), occipital carina joining with hypostomal carina at

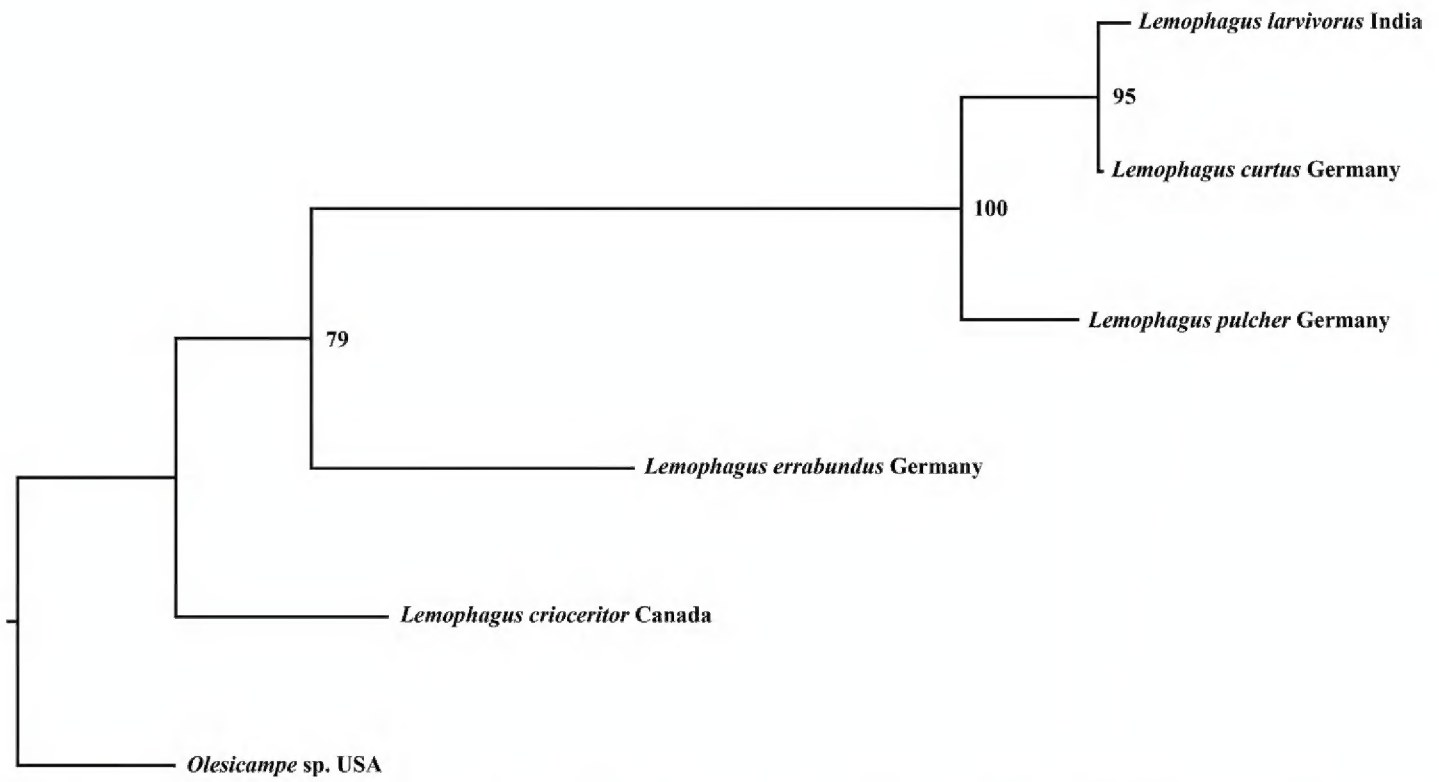

Figure 3. Maximum Likelihood tree of the *Lemophagus* species based on COI+28S gene fragments. Bootstrap values are provided at the nodes.

Figure 4. Biology of *Lemophagus larvivorus* sp. nov. **A** Larva of *Crioceris nigroornata* on asparagus plant **B, C** parasitized larva of *C. nigroornata*.

base of mandible (above base of mandible in *L. curtus*), mid-longitudinal carina of frons with transverse striae (without transverse striae in *L. curtus*), speculum coriaceous (smooth and shiny in *L. curtus*) and mesoscutum transversely striate posteriorly (uniformly rugose-punctate in *L. curtus*). Additionally, the new species exhibited similarity with *L. pulcher* but can be distinguished from *L. pulcher* by the following differences; upper tooth of mandible slightly longer than lower tooth (with same length in *L. pulcher*), clypeus punctate (rugose in *L. pulcher*) and speculum coriaceous (smooth in *L. pulcher*). The new species can be distinguished from *L. japonicus* by the following characters; malar space 0.8 × as long as basal width of mandible (as long as basal width of mandible in *L. japonicus*), area basalis of propodeum rectangular (triangular in *L. japonicus*) and hind tibia reddish brown apically (black in *L. japonicus*). The new species can be distinguished from *L. nanus* by the following differences; propodeum with costula (without costula in *L. nanus*), upper tooth of mandible slightly longer than lower tooth (with same length in *L. nanus*), speculum coriaceous (smooth and shiny in *L. nanus*) and clypeus punctate with smooth interspace (punctate with granulate interspace in *L. nanus*).

Molecular results

Of the ten described species, molecular data were available for four. The combined maximum likelihood analysis of COI + 28S showed that *L. larvivorus* was recovered as a distinct lineage from *L. curtus* with 95% ultrafast rapid bootstrap support (Fig. 3). Based on molecular data, *L. larvivorus* sp. nov. is related to *L. curtus*, and both species form a separate clade, with *L. pulcher*, *L. errabundus* and *L. crioceritor* as sister groups (Fig. 3; Suppl. material 2).

Parasitism of *Crioceris nigroornata*

Lemophagus larvivorus sp. nov., was recorded as the predominant parasitoid of *C. nigroornata*, with a mean parasitism rate of 12.5%. Additionally, an unidentified tachinid fly was also recovered, contributing to a mean parasitism rate of 5.0%.

Discussion

The campoplegine genus *Lemophagus* is considered a specialist larval endoparasitoid of criocerine beetles (Haye and Kenis 2004; Broad et al. 2018). Most of the host associated information is available from the Palaearctic region (Gold et al. 2001; Salisbury 2003; Haye and Kenis 2004). Present study extended the host association of *Lemophagus* from the criocerine genus, *Crioceris* as three species of *Lemophagus* were reported from this host genus (*L. crioceritor*, *L. errabundus* and *L. pulcher*) (Hendrickson et al. 1991; Gold et al. 2001; Salisbury 2003; Haye and Kenis 2004). Most of the *Lemophagus* species are associated with *Lilioceris* spp. whereas species like *L. errabundus* and *L. pulcher* have been reported from *Lilioceris* and *Crioceris* (Yu et al. 2016). Parasitoid

complex of lily leaf beetle (*Lilioceris* spp.), cereal leaf beetle (*Oulema* spp.) and asparagus beetle (*Crioceris* spp.) found to be similar (Gold et al. 2001; Haye and Kenis 2004). It can be assumed most of the *Lemophagus* are specific to the host groups. But based on the presence of more generalist *Lemophagus* species (*L. errabundus* and *L. pulcher*) the chances of expanding host parasitoid association of *Lemophagus* from the economically important criocerine genera.

Lemophagus larvivorus sp. nov. was recovered as the predominant parasitoid of *C. nigroornata*, with a mean parasitism rate of 12.5% during October 2020. In addition, an unidentified tachinid fly (Diptera: Tachinidae) was also recovered with a parasitism rate of 5.0%. Similarly, Hendrickson et al. (1991) reported *Lemophagus crioceritor* Aubert (Hymenoptera: Ichneumonidae) as a parasitoid of the asparagus beetle (*Crioceris asparagi* L.), with a parasitization rate of 7.9%. Our study confirms the parasitization of *C. nigroornata* by *L. larvivorus*, adding to the existing knowledge of parasitoid-host interactions in asparagus crops. This host record increases the possibility for using the parasitoid as a potential biocontrol agent against pests attacking asparagus. It also contributes to the understanding of natural enemy dynamics and potential biological control strategies for asparagus beetles.

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Supplementary material 1

Concatenated dataset (COI+28S) used in the phylogenetic analysis

Authors: A.P. Ranjith, Santosh C. Kedar

Data type: txt

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Link: https://doi.org/10.3897/jhr.98.154760.suppl1

Supplementary material 2

Output maximum likelihood tree from IQTREE

Authors: A.P. Ranjith, Santosh C. Kedar

Data type: treefile

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